Al-Qadisiyah Journal of Veterinary Medicine Sciences
(P-ISSN 1818-5746/ E-ISSN 2313-4429)
www.qu.edu.iq/journalvm

Research article

Molecular detection and phylogenetic analysis of cutaneous fibro papillomatosis in Iraqi Buffalo

Khalefa Ali Mansour Dhafer Rasheed Al-Fetly Salah M. Karim Raed Khat Majal
Department of Internal Medicine, Colege. of Veterinary Medicine, University of Al-Qadisiyah, Iraq.
Corresponding Author Email: Khalefa.mansor@ qu.edu.iq

Abstract:

Bovine papillomatosis (BP) is conveyed worldwide among cattle, however, is moderately less regular in water buffalo. The point of the present work was Molecular detection of cutaneous fibro papillomatosis in Buffalo in Al-Qadisiyah province, Iraq. Forty cutaneous warts (CW) samples were collected from lesions distributed on any part of the body (shoulder, thigh, around the eye region, and in the perineal region) of buffaloes in different ages. Out of 40 samples subjected to PCR, 26 samples were positive for BPVs. From the 26 positive samples, 4 samples were sent for DNA sequence analyses and found two samples was positive for Deltapapillomavirus (BPV-1) and two samples positive for Xipapillomavirus (BPV-4). The sequencing analysis for the BPV L1 fragment was identified from the CW that shown a 100% identity with BPV-4 from Iraq (KY662042.1_1) and BPV-1 sequences from Poland (KX594402.1 and KF284141.1). The molecular and phylogenetic results of the current study reported the similarity and close relation between the BPV-1 and 4 genotypes of bovine and buffalo in Iraqi breeds and other BPV strains from other countries.

Keywords: **Buffalo; Iraq; Molecular; Papillomatosis; Phylogenetic tree**

Introduction:

There are multiple tumors (benign and malignant) as cutaneous papilloma, benign fibroplasias and esophagus cancer are caused by the double-stranded circular DNA bovine papillomavirus (BPV) (1). The Bovine papilloma virus is a responsible for a significant economic cases due to weight loss, decrease milk production and growth retardation (2). Based on homology of the most conserved sequence genomic regions of the L1 ORF, the BPV are classified into three genera (3). Delta papillomavirus (BPV-1, 2 and 13), Epsilon papillomavirus (BPV-5 and 8) Papillomavirus (BPV-3, 4, 6, 9, 10, 11 and 12) and BPV-7 that remains not involved in any genre (4). The first molecular detection of bovine papillomavirus type 1 (BPV-1) in Iraq was recorded by (5). BPV is a commonly oncogenic virus in both dairy and beef Iraqi cattle, resulting in heavy economic losses. BPV in Iraq is related with the development of both benign and malignant warts. The main histopathological lesions type a fibropapilloma and the main causative agent a BPV-1(5). The BPV lesions most commonly found on the head, neck, udder, teats, legs, back and abdomen, as well as histopathological feature of these lesions include papillary projections (epidermal and dermal interdigitating) as well as fibroblast proliferation, collagen deposition, and lymphocyte infiltration (5).

Materials and Methods:

Ethical approval

The present study was approved by The Ethical Committee of College of Veterinary Medicine, University of Al-Qadisiyah, Iraq.

Samples collection

Skin wart samples were collected from different papilloma lesions that were

Al-Qadisiyah Journal of Veterinary Medicine Sciences
(P-ISSN 1818-5746/ E-ISSN 2313-4429)
www.qu.edu.iq/journalvm

distribution on the many part of the body (shoulder, thigh, around the eye region, and in the perineal region). These samples were collected from buffaloes in different ages and were expected to be clinically diagnosed as warts. The samples was cut by a sterile blade in small container and then immediately transported to lab under cold condition until the PCR assay were done.

DNA viral extractions

Viral genomic extraction from icy skin samples (skin wart lesion) according geneaid company, USA, genomic (DNA Mini kit extraction). The steps of DNA extraction were done according to company instructions, by way of proteinase K. Concentrated DNA concentrations were measured by Nanodrop, All DNA concentrations obtained within the required level.

Primers

PCR amplification: the PCR based done of papillomatosis (BPV-1) in water buffaloes using specific primers used for detection L 1-gene of major capsid protein .(F- CGGGGCCAAAACTGTTCCTA) (R-AATTCAAGAGGAGGGCAGGC) at (399) bp, this primers were design according to (6).

PCR assay condition

The PCR master mix was carried out on the word of the manufacturer's instructions, Bioneer. Korea, PCRpreMIxkit (Accu Power). Master mix tube comprises from pellet (freeze–dried) of dNTPs (250µM), Tag DNA polymerase 1U, Tris HCL 10 µM(at pH 9.0), MGCL(1.5) µM , KCL(30 µM), tracking dye and stabilizer. The PCR reaction was set based to kit direction in 20 µM of whole volume by added 5 µM of DNA as well as mixing 3 µM of the primers by 1.5 of each forward and revers. Then the PCR Premix tub was completed to 20 µM by added PCR water. The whole contains were maxing by vortex centrifuge. The thermocyler condition was done as the following steps: the initial denaturation for 5 min at (95° C); thirty cycles of denaturation for 30 Sec. at 95° C, annealing 58° C for 30 Sec. and extension for 30 Sec. at 72 °C and final extension for 5 min. at 72° C. The result PCR ends products were tested using electrophoresis in agrose gel (1%) and seen under U.V light. The end product was performed to DNA sequencing (AB, DNA sequencing methods system). Nucleotide sequence were ready for alignment and present of identity of local isolates strains with other selected world isolates using NCBI, Clustal W W2 program online. The sequences achieved in this work subjected to phylogenetic analysis.by using MEGA6 software in version0.6 to make present of identity and relationship between examined sequences and also achieved phylogenetic tree study (7).

Results:

The cutaneous warts were observed in the many cows and buffaloes herds and included herds grown in organized breeding and those raised by farmers in small groups in Al-Qadisiyah Province. By using definite primers to BPV-1, L1 gene of major capsid protein gene at 399 bp amplicon. Out of forty CW samples of buffaloes send to PCR, 26 samples were positive for BPVs. Figure (1). The end PCR products of amplicon of positive result was that were sequencing and come to confirm the PCR result of genotyping of the BPV type-1 that affected buffalo which isolated from deferent area of Al-Qadisiyah Province. The PCR positive samples were sent for DNA sequencing. From the 26 positive samples 4 samples were sent for DNA analysis and found 2 sample was positive for *Deltapapillomavirus* (BPV-1) and 2 positive for *Xipapillomavirus*(BPV-4). The sequencing analysis of the BPV L1 fragment identified from the cutaneous wart shown a 100% identity with BPV-4 from Iraq (KY662042.1_1) and 99% BPV-1 sequences from Poland (KX594402.1 and KF284141.1) Figure (2).

Al-Qadisiyah Journal of Veterinary Medicine Sciences
(P-ISSN 1818-5746/ E-ISSN 2313-4429)
www.qu.edu.iq/journalvm

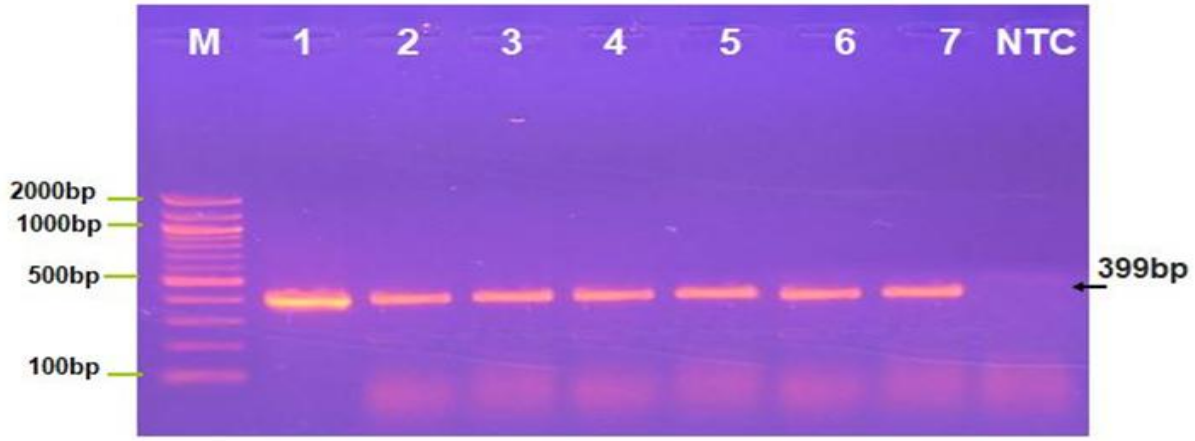

Figure (1). PCR product for (BPV-1/ L1gene at 399bp) that visualized Agrose gel (1.5%) lane 1,2,3,4,5and 6 represents positive to, M marker and NTC negative control.

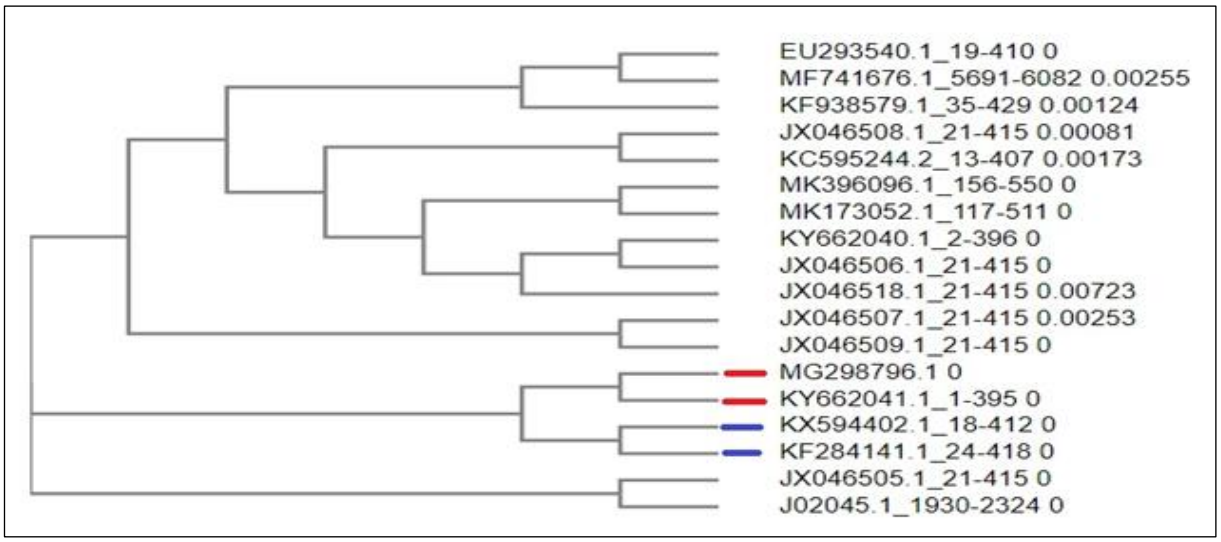

Figure (2): Phylogenetic tree of Iraqi isolates (partial genome) for bovine papilloma virus (type-1) with other world reference using a Mega 6. The read lines (Our Iraqi isolate MG MG298796.1_0 and another Iraqi isolate KY662042.1_1). The blue lines (Poland isolates KX594402.1 and KF284141.1).

Discussion:

The BPV-1 Iraqi strain was identified in a different sites (shoulder, thigh, around the eye region, and in the perineal region). The use of specific primers 399 bp (F-CGGGGCCAAAACTGTTCCTA) (R-AATTCAAGAGGAGGGCAGGC) for detection L 1-gene of major capsid protein has also established the reality of a great diversity of BPV types (8, 9). Papilloma virus look to be constant genetically and the incidence of mutation or recombination, is an unusual (10). The identification of BPV-1 in Asia (China, India and Iraq), Europe (Poland, Croatia and Turkey), and Brazil, suggests the earlier presence of this BPV type in these countries (11). It is potential that the occurrence of BPV-1 in Iraq was ignored (12). The results of our study submits the low diversity detected in BPV (BPV-1 to BPV-4) because of the little quantity of viruses observed more willingly than because of a little diversity in the BPV genome (13,

Al-Qadisiyah Journal of Veterinary Medicine Sciences
(P-ISSN 1818-5746/ E-ISSN 2313-4429)
www.qu.edu.iq/journalvm

14). Furthermore, the revealing of this kind in Iraq point to its potential spreading through Iraqi buffaloes. The latest studies in BPV underline the significance of new surveys including the molecular epidemiological studies of BPV infections in cattle of the world (15). A consensus sequence of 5484–5933 and 6293–6753 of BPV-3(GenBank accession number MG298796) was obtained with the BAA (1-5) and BAPV (1-10) and the last region was used for phylogenetic tree analysis of BAPV6MY and BAPV11MY. Nevertheless, our Iraqi BPV isolate was noticed in a skin wart located in the axillary region of a buffalo and was thus accompanying by cutaneous papillomatosis (16, 17). According to the phylogenetic analysis; our Iraqi isolate MG298796.1_0 and KY662042.1_1 of BPV were fairly similar in nucleotide sequence and prepared in the same tree node and clustered together closely with whole similarity in sequence of nucleotides, as well there was some sequential difference and similarities between those of the Polish isolates KX594402.1 and KF284141.1 (6).

Conclusion:

In present study, the bovine papillomatosis cases were clinical and histological diagnosed in water buffalo to recognize and characterize the BPV-1 genotypes which were the most predominant in the Iraq. The molecular and phylogenetic results of the current study reported the similarity and close related between the BPV-1 and 4 genotypes of bovine and buffalo in Iraqi breeds. The identification and characterization of the BPV-1and 4 genotypes present in this region are essential for real control of disease. More investigations include the BPV genotyping in water buffalo are needed to confirm the data of current study.

Acknowledgements:

We are very grateful to the Internal Veterinary Dept., Coll of Veterinary Medicine, University of Al-Qadisiyah. Furthermore, we thank the owners of the buffalo herds for giving us consent to examine their herds and take the necessary samples. The study was self-funded.

Competing interests

The authors declare that they have no competing interest.

References:

1-Campo M. Papillomavirus and disease in humans and animals. Veterinary and Comparative Oncology 2003; 1, 3-14.

2-Santos E, Silva M, Pontes N, Coutinho L, Paiva S, Castro R, Freitas A. Detection of different bovine papillomavirus types and co-infection in bloodstream of cattle. Transboundary and emerging diseases 2016; 63, e103-e108.

3-de Villiers E.-M. Cross-roads in the classification of papillomaviruses. Virology 2013; 445, 2-10.

4-Araldi R, Melo T, Diniz N, Mazzuchelli-de-Souza J, Carvalho R, Beçak W, Stocco R. Bovine papillomavirus clastogenic effect analyzed in comet assay. BioMed research international 2013.

5-Hamad MA, Al-Shammari AM, Odisho SM, Yaseen NY. Molecular and phylogenetic analysis of bovine papillomavirus type 1: First report in Iraqi Cattle. Advances in virology 2016.

6-Khalefa AM. Clinical, histopathological and molecular detection of cutaneous papillomatosis associated with bovine papillomavirus types 1 and 2 in cattle from Al-Qadisiyah Governorate. Kufa J Vet Med Sci., 2016; 7(1):182-191.

7-Tamura K, Stecher G, Peterson D, Filipski A, Kumar S. MEGA6: molecular evolutionary genetics analysis version 6.0. Molecular biology and evolution 2013; 30, 2725-2729.

8-Maeda Y, Shibahara T, Wada Y, Kadota K, Kanno T, Uchida I, Hatama S. An outbreak of teat papillomatosis in cattle caused by bovine papilloma virus (BPV) type 6 and unclassified BPVs. Veterinary microbiology 2007; 121, 242-248.

9-Ogawa T, Tomita Y, Okada M, Shinozaki K, Kubonoya H, Kaiho I, Shirasawa H. Broad-spectrum detection of papillomaviruses in bovine teat papillomas and healthy teat skin. Journal of general virology 2004; 85, 2191-2197.

10-Claus MP, Lunardi M, Alfieri AF, Sartori D, Fungaro MHP, Alfieri AA. Identification of the recently described new type of bovine papillomavirus (BPV-8) in a Brazilian beef cattle herd. Pesquisa Veterinária Brasileira, 2009; 29(1), 25-28.

11-Trewby H, Ayele G, Borzacchiello G, Brandt S, Campo MS, Del Fava C, Marais J, Leonardi L,

Al-Qadisiyah Journal of Veterinary Medicine Sciences
(P-ISSN 1818-5746/ E-ISSN 2313-4429)
www.qu.edu.iq/journalvm

Vanselow B, Biek R. Analysis of the long control region of bovine papillomavirus type 1 associated with sarcoids in equine hosts indicates multiple cross-species transmission events and phylogeographical structure. The Journal of general virology 2014; 95, 2748.

12-Nasir L, Campo MS. Bovine papillomaviruses: their role in the aetiology of cutaneous tumours of bovids and equids. Veterinary dermatology 2008; 19, 243-254.

13-Chan SY, Delius H, Halpern AL, Bernard HU. Analysis of genomic sequences of 95 papillomavirus types: uniting typing, phylogeny, and taxonomy. Journal of virology, 1995; 69(5), 3074-3083.

14-Dagalp SB, Dogan F, Farzanı TA, Salar S, Bastan A. The genetic diversity of bovine papillomaviruses (BPV) from different papillomatosis cases in dairy cows in Turkey. Archives of virology, 2017; 162(6), 1507-1518.

15-Silvestre O, Borzacchiello G, Nava D, Iovane G, Russo V, Vecchio D, D'Ausilio F, Gault EA, Campo MS, Paciello O. Bovine papillomavirus type 1 DNA and E5 oncoprotein expression in water buffalo fibropapillomas. Vet Pathol. 2009;46:636-641.

16-Leishangthem GD, Somvanshi R, Tiwari AK. Detection of bovine papillomaviruses in cutaneous warts/papillomas in cattle. Indian J. Vet. Pathol, 2008; 32(1), 15-20.

17-Jangir BL, Bind RB, Kumar P, Somvanshi R. Pathological studies and detection of different bovine papilloma virus types in buffalo cutaneous warts. Turkish Journal of Veterinary and Animal Sciences, 2017; 41(2), 306-311.